

WHAT IS CLAIMED IS:

1. Method for the preparation of crosslinked enzyme aggregates, comprising the steps of:

A - providing a plurality of enzyme molecules,

B - aggregating the enzymes in a liquid medium, comprising a precipitating agent,

C - crosslinking the aggregated enzymes to one another by providing a crosslinking agent in the liquid medium, wherein the crosslinking agent being prepared by combining a first and a second compound each having at least two reactive groups, the reactive groups of the first compound being primary amino groups, the reactive groups of the second compound being aldehyde groups.

2. Method according to claim 1, wherein the first compound comprises at least two carbon atoms, the termini of the backbone being defined as  $\alpha$  and  $\omega$ , respectively, the said termini both comprising the active groups.

3. Method according to claim 1, wherein the first compound is chosen from the group consisting of diaminoalkanes, triamines, aromatic diamines, diamines having at least one hetero atom between the amino groups, branched diamines or a combination of two or more thereof.

4. Method according to claim 1, wherein the second compound is a dialdehyde.

5. Method according to claim 1, wherein the second compound is chosen from the group, consisting of glutaraldehyde, glyoxal, 2,3-pentadione, 2,4-pentadione, 2,4-hexadione, 3,4-hexadione, 3-methyl-2,4-pentadione, 3-ethyl-2,4-pentadione, or a combination of two or more thereof.

6. Method according to claim 1, wherein the crosslinking agent is prepared in a substantially protein free environment.

7. Method according to claim 1, wherein the second and the first compound are combined in a molar ratio of 10-1:1, preferably 4-1:1, more preferably of 2,5-1,5:1, most preferably of 2:1.
8. Method according to claim 1, wherein the enzyme molecules are chosen from lipases, esterases, proteases, nitrilases, oxynitrilases, penicillin amidases and amino acylases.
9. Crosslinked enzymes aggregate, obtainable by claim 1.
- ~~10.~~ Crosslinking agent prepared by combining a first compound comprising at least two carbon atoms, the termini of the backbone being defined as  $\alpha$  and  $\omega$ , respectively, the said termini both comprising the active groups, the second compound being a dialdehyde.
11. Crosslinking agent according to claim 10, wherein the first compound is chosen from the group consisting of diaminoalkanes, triamines, aromatic diamines, diamines having at least one hetero atom between the amino groups, branched diamines or a combination of two or more thereof.
12. Crosslinking agent according to claim 10, wherein the second compound is chosen from the group, consisting of glutaraldehyde, glyoxal, 2,3-pentadione, 2,4-pentadione, 2,4-hexadione, 3,4-hexadione, 3-methyl-2,4-pentadione, 3-ethyl-2,4-pentadione, or a combination of two or more thereof.
- ok 13. Use of a crosslinking agent according to claim 10 for crosslinking a protein to another protein or a carrier, preferably a solid carrier.